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# Reciprocal relationship between contact and complement system activation on artificial polymers exposed to whole human blood

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ABSTRACT

*Background:* Inappropriate and uncontrolled activation of the cascade systems in the blood is a driving force in adverse inflammatory and thrombotic reactions elicited by biomaterials, but limited data are available on the activation of the contact system by polymers and the present study was undertaken to investigate these mechanisms in established models.
*Methods:* Polymer particles were incubated in (1) EDTA-plasma (10 mM) to monitor the adsorption of 20 selected proteins; (2) lepirudin-anticoagulated plasma to evaluate contact system activation, monitored by the formation of complexes between the generated proteases factor[F]XIIa, FXIa and kallikrein and the serpins C1-inhibitor [C1INH] and antithrombin [AT]; (3) lepirudin-anticoagulated whole blood to determine cytokine release.
*Results:* Strong negative correlations were found between 10 cytokines and the ratio of deposited FXII/C1INH, generated FXIIa-C1INH complexes, and kallikrein-C1INH complexes. Formation of FXIIa-C1INH complexes correlated negatively with the amount of C3a and positively with deposited IgG.
*Conclusions:* A reciprocal relationship was found between activation of the contact system and the complement system induced by the polymers studied here. The ratios of FXII/C1INH or C4/C4BP, adsorbed from EDTA-plasma are useful surrogate markers for cytokine release and inflammatory response to materials intended for blood contact.



## 1. Introduction

Medical devices used in the clinic contain materials alien to the body that will trigger activation of the innate immune system when they make contact with the blood, a situation that may occur either transiently during implantation or during the whole active lifetime of the device. The cascade systems of the blood; contact activation/coagulation, and the complement and fibrinolytic systems, all function according to similar principles: recognition molecules target non-self structures, leading to the generation of various mediators, which in turn activate the platelets, monocytes, and granulocytes, and ultimately lead to inflammation and thrombosis. We have described a model for the sequence of events in this process, in which the composition and conformation of proteins in the initial layer that is formed when a surface makes blood contact is of critical importance, and that subsequent reactions are dependent on the crosstalk between the different cascade and cellular systems in the blood [1–5].

In order to dissect these reactions in a systematic way, we synthesized six polymers with different physico-chemical

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properties and characterized these compounds as well as reference compounds of polystyrene (PS), polyvinylchloride (PVC), and glass regarding their interactions with plasma proteins. The six test polymers were synthesized using different monomers and cross-linkers as described previously by co-polymerization of the monomer and crosslinker (both given in Table 1) using thermally initiated free radical polymerization with AIBN (1 mol%) in ethanol, or APS (1.6 mol%) in combination with TEMED (0.8 mol%) in water. Thereafter, the polymer monoliths, designated P1 – P6, were ground to particles (25–63 μm) and were characterized together with control particles using an array of physico-chemical techniques as summarized in Table 1 [6].

We then studied the subsequent activation and regulation of the complement system, which leads to leukocyte activation, and ultimately results in the release of a number of cytokines. This approach, using blood systems of increasing complexity (EDTA-plasma [10 mM final concentration], lepirudin-anti-coagulated plasma, and whole blood) allowed us to define the ratio of adsorption of the complement component C4 and its inhibitor, C4 binding protein [C4BP], as predictors of an inflammatory response [6,7].

The scope of the present work was to apply these studies of material-induced cascade system activation to investigate the contact system, using the same, well-characterized polymer particles. The contact system consists of the zymogen molecules factor [F] XII, FXI, and prekallikrein [PK], and of high molecular weight kininogen [HK], which acts as a co-factor for both FXI and PK. FXII has an affinity for negatively charged compounds and becomes autoactivated to α–FXIIa upon binding. The surface-bound α–FXIIa can cleave soluble FXII to β–FXIIa. It in turn activates FXI which is in complex with HK, thereby initiating the coagulation cascade, leading to subsequent thrombin formation and clotting. Of further importance is the fact that α–FXIIa, via β–FXIIa, can activate PK (which is also in complex with HK) to kallikrein [KK], which acts as a positive regulator by activating more FXII molecules to soluble β-FXIIa, generating more KK. Furthermore, KK also cleaves HK, which results in the release of the potent vasoactive and proinflammatory nonapeptide bradykinin [8]. In addition, contact system activation has been demonstrated to occur upon interaction with activated endothelial cells [9,10] and activated platelets [11,12], as well as on the surface of fibrin clots [13].

The generated proteases of the contact system (FXIIa, FXIa, and KK) are inhibited by the serine protease inhibitors [serpins] C1 inhibitor [C1INH] and antithrombin [AT]. This activation/inhibition process can be visualized by detecting the resulting protease–serpin complexes: FXIIa-C1INH, FXIa-C1INH, KK-C1INH, FXIIa-AT, FXIa-AT, and KK-AT, respectively. C1INH has traditionally been regarded as the predominant inhibitor of the contact system proteases [14], and by using protease–serpin complex assays, we have demonstrated that this is indeed the case when activation has been initiated by negatively charged surfaces such as glass or kaolin [15]. In contrast, in situations in which the contact system is activated following platelet activation or is triggered by preformed fibrin clots, protease-AT complexes but almost no protease-C1INH complexes are detected [12,13,15].

Here we have extended our earlier studies of protein fingerprinting on the polymer surfaces from human EDTA-plasma to also include the components of the contact/coagulation system. In addition, we have monitored the activation of the contact system that is induced by the previously characterized polymers after incubation in plasma anticoagulated with the specific thrombin inhibitor lepirudin, followed by detection of the various protease–serpin complexes formed, and demonstrated firm correlations between these events and subsequent inflammatory response to the same polymers. These new results, combined with those of our previous studies, have enabled us to construct a simple, robust protocol for biomaterial testing, based on differential adsorption of four key proteins, (FXII, C1INH, C4 and C4BP) to surfaces exposed to EDTA-inactivated human plasma. At present there is a shortage of simple biocompatibility test systems so we envisage that this protocol will become useful at various sites of biomaterial development and research.

## 2. Methods

### 2.1. Synthesis and physico-chemical characterization of polymers

Six polymers were synthesized using different monomers and cross-linkers as described previously [6]. The novel polymers, designated P1 – P6, were ground to particles (25–63 μm) and were characterized together with control particles of PS, PVC, and glass, using an array of physico-chemical techniques as summarized in Table 1 [6,7].

### 2.2. Blood and plasma preparations

Blood was drawn from subjects registered as blood donors at local the Blood Banks in Sweden thus fulfilling the health requirements defined in the EDQM Guide 18th edition [16]. In addition, it was ascertained that they had not taken any medications (e.g. ASA) known to interfere with platelet function, or any anticoagulantia, for at least 10 days prior to phlebotomy. The blood was collected in Vacutainer™ tubes (Becton, Dickinson and Co., Plymouth, UK) containing the specific thrombin inhibitor lepirudin (Refludan, Celgene Europe Ltd, Windsor, UK), final concentration 50 μg/mL), and either used immediately or centrifuged at 2450 × g for 25 min at 4 °C to obtain plasma.

In addition, plasma containing 10 mM EDTA from five different donors was pooled. The study was performed with the consent of the Ethical Committee of the University of Linköping, Sweden (#03–520).

**Table 1**
Composition, zeta-potential, surface area, pore size, and density and functional groups of polymers P1–P6 and reference polymers, PS, PVC, and glass characterized in Refs. [6,7].

| | Monomer/crosslinker (20%:80%) | Zeta-potential (mV) | | Surface area ($m^2/g$) | Pore size (Å) | Density ($g/cm^3$) | Functional group |
|---|---|---|---|---|---|---|---|
| P1 | MAA/DAP | 2.37 ± 1.43 | Hydrophilic | 188 | 133.5 | 1.2 | $-COO^-$ |
| P2 | MAA/DBV | −24.06 ± 3.46 | Hydrophobic | 161 | 27.8 | 0.9 | $-COO^-$ |
| P3 | MAA/EGDMA | −15.10 ± 2.06 | Hydrophobic | 203 | 84.4 | 1.2 | $-COO^-$ |
| P4 | IPAAm/EGDMA | −14.28 ± 1.93 | Hydrophobic | 130 | 32.1 | 1.2 | $-CONH_2CH(CH_3)_2$ |
| P5 | Styrene/EGDMA | −16.43 ± 0.70 | Hydrophobic | 25 | 101.6 | 1.2 | $-C_6H_5$ |
| P6 | HEMA/EGDMA | −11.74 ± 0.29 | Hydrophobic | 105 | 116.6 | 1.2 | −OH |
| PS | Styrene | −16.96 ± 1.98 | Hydrophobic | 11 | 45.4 | 1.1 | $-C_6H_5$ |
| PVC | Vinyl Chloride | −8.54 ± 1.82 | Hydrophobic (less) | 17 | 40.9 | 1.4 | −Cl |
| Glass | | −20.16 ± 0.98 | Hydrophilic | 6 | 512 | 2.5 | −SiOH/SiO(−) |

*N,N*-diacryloylpiperazine (DAP); divinylbenzene (DVB); ethylene glycol dimethacrylate (EGDMA); methacrylic acid (MAA); *N*-isopropyl acrylamide (IPAAm); 2-hydroxyethyl methacrylate (HEMA); polystyrene (PS); polyvinylchloride (PVC).